

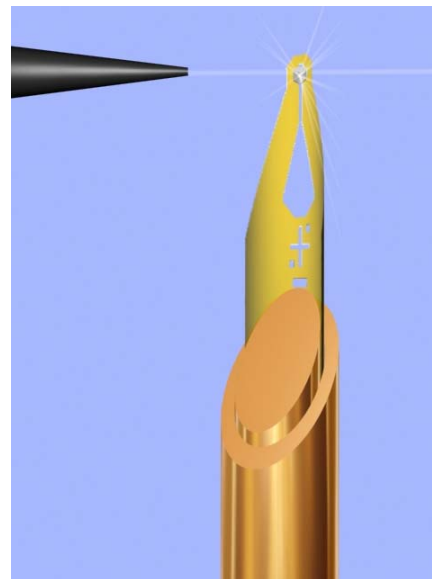
MicroMounts: A New Tool for Structural Genomics

R. E. Thorne, Cornell University and MiTeGen, LLC

DMR-0405500

An understanding of the structure of proteins and other biological macromolecules is essential for fundamental understanding of disease and for the development of new medicines. We have developed new tools for retrieving, manipulating and mounting crystals of both small molecules and proteins for molecular structure determination by X-ray crystallography. These microfabricated tools are ideally suited to handle the very small (<50 micrometer) crystals typically produced in high-throughput structural genomics efforts, and allow easy automated crystal alignment and data collection at synchrotron beam lines. They are now being widely adopted by the structural genomics community.

J. Appl. Cryst. **36**: 1455-1460 (2003).



A protein crystal held by a MicroMount™ holder and illuminated by an X-ray beam. The mount consists of a thin microfabricated, X-ray transparent film attached to a stainless steel post. These mounts resolve all of the problems associated with the current cryoloop method, and have been used at microfocus synchrotron beam lines to determine protein structures using sub-10 micrometer crystals.

One of the most serious problems in determining the structures of proteins and other biological macromolecules is obtaining crystals of sufficient size and quality for X-ray crystallographic studies. High-throughput robotic expression, purification and crystallization methods have helped to increase the rate at which crystals can be obtained, but most of these crystals are quite small. Furthermore, many of the most interesting targets for structure determination are protein-protein complexes and protein-nucleic acid complexes, whose crystals also tend to be quite small. These small crystals are difficult to handle with the cryoloop method now used almost exclusively by the structural biology community. This method has several other problems that reduce the quality of the molecular structures obtained and that make automated crystal alignment in the X-ray beam difficult.

Beginning with a one-semester independent study project by undergraduate Adam Saltzman, we have developed a microfabricated alternative to cryoloops that resolves all of their problems and that is ideally suited to handling very small crystals and to automated alignment. Kevin O'Neill, an NSF-supported graduate student, developed our basic microfabrication processes at the NSF-funded Cornell Nanofabrication Facility. These process were elaborated upon by Zachary Stum, initially supported by NASA and now supported by a Department of Education Fellowship, and further design and process evolution has been performed by Adam Bartnik, supported by the NIH. Mariya Bessonov, a mathematics undergraduate from North Carolina State, also assisted as an NSF-supported REU student by setting up finite element simulations to optimize the microfabricated film's stiffness while minimizing its thickness and X-ray absorption/scattering. More than 2000 of these tools were distributed to the structural genomics community in 2003-2004 with support from the NIH. During this time, the PI worked with Cornell and potential licensees to make these tools commercially available. When it became clear that none of the potential licensees had sufficient expertise to continue developing these tools to meet the community's evolving needs, the PI established his own company, MiTeGen, LLC (www.mitegen.com). Ongoing development and prototype production is being performed at the NSF-funded CNF, and assembly, packaging and shipping is being performed at Cornell's industrial park.

R. E. Thorne, Cornell University and MiTeGen, LLC

DMR-0405500

Education:

Three undergraduate students (Adam Saltzman, now a graduate student at Duke, Mariya Bessonov, an REU student from NC State, and Adam Bartnik, now a graduate student at Cornell), two graduate students (Kevin O'Neill, now a postdoctoral associate at the Technical University of Delft, and Zachary Stum) and one postdoctoral associate (Jan Kmetko) have been involved in developing these mounts. O'Neill, Stum and Kmetko are named with the PI on a pending patent application.

Societal Impact:

The PI started a company - MiTeGen, LLC (www.mitegen.com) - in May 2004 to manufacture and distribute these tools to the structural genomics community. Initial response in North America, Europe and Japan has been enthusiastic. These tools seem likely to largely supplant the current technology, and dramatically simplify structure determination using very small crystals. They will increase the rate at which protein structures are solved and the quality of those structures, and thus speed the development of new medicines.